

Emergence and initial growth of *Schizolobium parahyba* var. *amazonicum* as a function of sowing depth in the amazon biome

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ABSTRACT: The direct seeds sowing reduces the costs of implanting of forest stand, but it is necessary to study the ideal sowing depth, since this factor can affect the emergence and initial growth of the seedlings. The aim of this study was to evaluate the influence of different sowing depths on emergence, survival, and initial growth of *Schizolobium parahyba* var. *amazonicum* in the field conditions. A randomized block design was adopted, with five treatments and five replications, of 20 seeds each. The treatments were different depth, as follows: 5 cm (T1), 10 cm (T2), 15 cm (T3), and 20 cm (T4). Were evaluated the percentage of emergence, average time and emergence speed index, collar diameter, number of leaves, aerial part height, degree of slenderness and survival rate of seedling at 120 days after sowing. The results showed that emergence started two days after sowing, stabilizing on the 13th day. The depths of 5, 10, and 15 cm showed satisfactory results in terms of emergence percentage, survival rate and initial growth. The sowing depth of 10 cm was the best results for the variables studied. The best result for the average time and emergence speed index were obtained at depth of 5 cm. Direct sowing of *S. parahyba* is a viable method to be implemented by small and medium farmers, aiming to reduce costs by eliminating the nursery phase. The ideal seeding depth for the emergence, survival and initial growth of *S. parahyba* is at a depth of 10 cm.

Key words: Amazon rainforest; direct seeding; forest seeds, Paragominas; Paricá

Emergência e crescimento inicial de *Schizolobium parahyba* var. *amazonicum* em função da profundidade de sementeira no bioma amazônico

RESUMO: A prática de sementeira direta reduz os custos de implantação de um povoamento florestal e para isso, faz-se necessário o estudo da profundidade ideal de sementeira, visto que esse fator pode afetar a emergência e o crescimento inicial das plântulas. O objetivo deste estudo foi avaliar a influência de diferentes profundidades de sementeira na emergência, sobrevivência e crescimento inicial de *Schizolobium parahyba* var. *amazonicum* em condições de campo. Adotou-se um delineamento em blocos casualizados, composto com cinco repetições de 20 sementes cada. Os tratamentos consistem em diferentes níveis de profundidade, sendo: 5 cm (T1), 10 cm (T2), 15 cm (T3) e 20 cm (T4). Avaliou-se a percentagem de emergência, tempo médio e índice de velocidade de emergência; diâmetro do coleto, número de folhas, altura da parte aérea, grau de esbeltez e a taxa de sobrevivência dos indivíduos aos 120 dias após a sementeira em campo. A emergência teve início 2 dias após a sementeira, estabilizando-se no 13º dia. As profundidades de 5, 10 e 15 cm demonstraram resultados satisfatórios quanto a porcentagem de emergência, taxa de sobrevivência e crescimento inicial. A profundidade de sementeira de 10 cm foi a que expressou os melhores resultados para as variáveis estudadas. Os melhores resultados para o tempo médio e índice de velocidade de emergência foi obtido na profundidade de 5 cm. A sementeira direta de *S. parahyba* é um método viável para ser implementado por pequenos e médios agricultores, visando a redução de custos com a eliminação da fase de viveiro. A profundidade de sementeira ideal para a emergência, sobrevivência e crescimento inicial do *S. parahyba* é de 10 cm.

Palavras-chave: Floresta amazônica; sementeira direta; sementes florestais; Paragominas; Paricá



Introduction

In the current scenario of planted forests, *Schizolobium parahyba* var. *amazonicum*, popularly known as Paricá, is a native species of the Amazon region of high commercial value, with a high potential for economic growth, being able to provide good products in planted forest systems, such as high-quality laminates, plywood, ceilings and matches (Silva et al., 2015; Noronha et al., 2018). Its rapid growth, straight stem and absence of knots in its wood, made it the sixth most planted tree species in Brazil, with about 88 thousand hectares of planted area in the states of North and Northeast regions of Brazil, Pará, Maranhão and Tocantins (Ibá, 2020).

The use of *S. parahyba* for purposes such as forest restoration or the use of wood in industry requires the production of seedlings, which depends exclusively on an efficient, high-quality training program (Binotti et al., 2019). The municipalities located in the southeast of the State of Pará are responsible for the implementation of large areas of plantations of the *S. parahyba* species for the purposes of timber industry commercialization, mainly to produce multi-laminated plywood panels and for the recovery of degraded areas (Gomes et al., 2021).

It is common in *S. parahyba* forest plantations the incorporation of seedlings in the field. These are formed under controlled conditions for approximately 45 days and taken to the field after seedling emergence and development (Gomes et al., 2021). In this process, according to variations in environmental conditions, the seedlings may suffer environmental stress when established in their final destination, which can generate mortality and thus increase the costs of replanting. The adoption of direct seeding as a silvicultural technique can be seen as a viable method in *S. parahyba* planting areas, considering that its use has advantages such as reduction of costs of implantation in labor and production of seedlings, and not moving the soil, which reduces environmental damage (Palma & Laurance, 2015; Gomes et al., 2021).

Direct sowing consists of introducing seeds of certain forest species directly into the soil of the area to be reforested. Direct sowing facilitates field implantation procedures and with a reduction in expenses when the results are satisfactory, eliminating the nursery phase, producing plants with a well-developed root system and without deformations. Thus, in practice, the ideal sowing depth is one that favors homogeneous seed germination, rapid seedling emergence and quality seedling production.

The exposure of seeds for long periods and inadequate depths can increase the susceptibility to pathogens. The superficial depths can result in the loss of seeds due to excess solar radiation on them, drying them and making their germination unfeasible, resulting in small and weak plants, in addition to making the seedlings easy targets for predators, causing damage to the radicle and damage caused by irrigation (Gehling et al., 2017). Thus, it is necessary to study before planting each species, aiming to save time and avoid

economic damage, based on the principle that each group has tolerances, individual characteristics (Teixeira et al., 2017).

For the direct sowing method, there is not enough information about the sowing depth of *S. parahyba* seeds for their development in the field. In order to optimize the planting phases and the development of the species in the field, it is essential to study the influence of different levels of depth for the viability of the direct sowing method, with the determination of the ideal sowing depth to reach a high percentage of germination, survival and satisfactory growth. Thus, the objective of this study was to evaluate the influence of different sowing depths on germination, survival, and initial development of *Schizolobium parahyba* var. *amazonicum* in the field.

Materials and Methods

Study area

The study was carried out in the experimental field of Embrapa Amazônia Oriental, located in the municipality of Paragominas, in the Southeast Mesoregion of the state of Pará, North region of Brazil, at the geographic coordinates 2° 59' 34.76" S latitude and 47° 24' 23.70" O of longitude (Figure 1A).

Paragominas is characterized by the occurrence of a hot and humid climate, of the Awi type according to the Köppen classification (tropical rainy climate, with a well-defined dry season). The annual temperature average of 26°C and an annual rainfall average of 1,700 mm, with the highest distribution in March. The soil was classified as Latossolo Amarelo distrófico (Brazilian classification, similar to Oxisol in the Soil Taxonomy and Ferralsols in the WRB), which predominates in more than 80% of the total area of the municipality. The medium and very clayey texture in flat and smooth wavy relief, with low fertility (Rodrigues et al., 2003).

The historic of experimental field was agricultural experimentation, with annual crops such as soybeans, corn and rice. Before implementing the experiment, was applied systemic herbicide (glyphosate) and selective herbicide (flumizym) to combat weeds, followed by harrow and leveling (Figure 1B).

Between January and February 2021, samples from 0-40 cm deep were collected to determine the chemical attributes of the soil (Teixeira et al., 2017). The soil analyses of the experimental area are presented in Table 1.

Experimental Design

A randomized block design was used, with four treatments and five replications. The treatments were four seeds sowing depths (5, 10, 15, and 20 cm), composed of 20 seeds for each treatment (Figure 1C). The experiment was installed in an area of 29.0 × 24.0 m (696 m²), divided into 25 plots of 5.0 × 4.0 m (20 m²), spaced 1.0 × 1.0 m between plots (Figure 4).

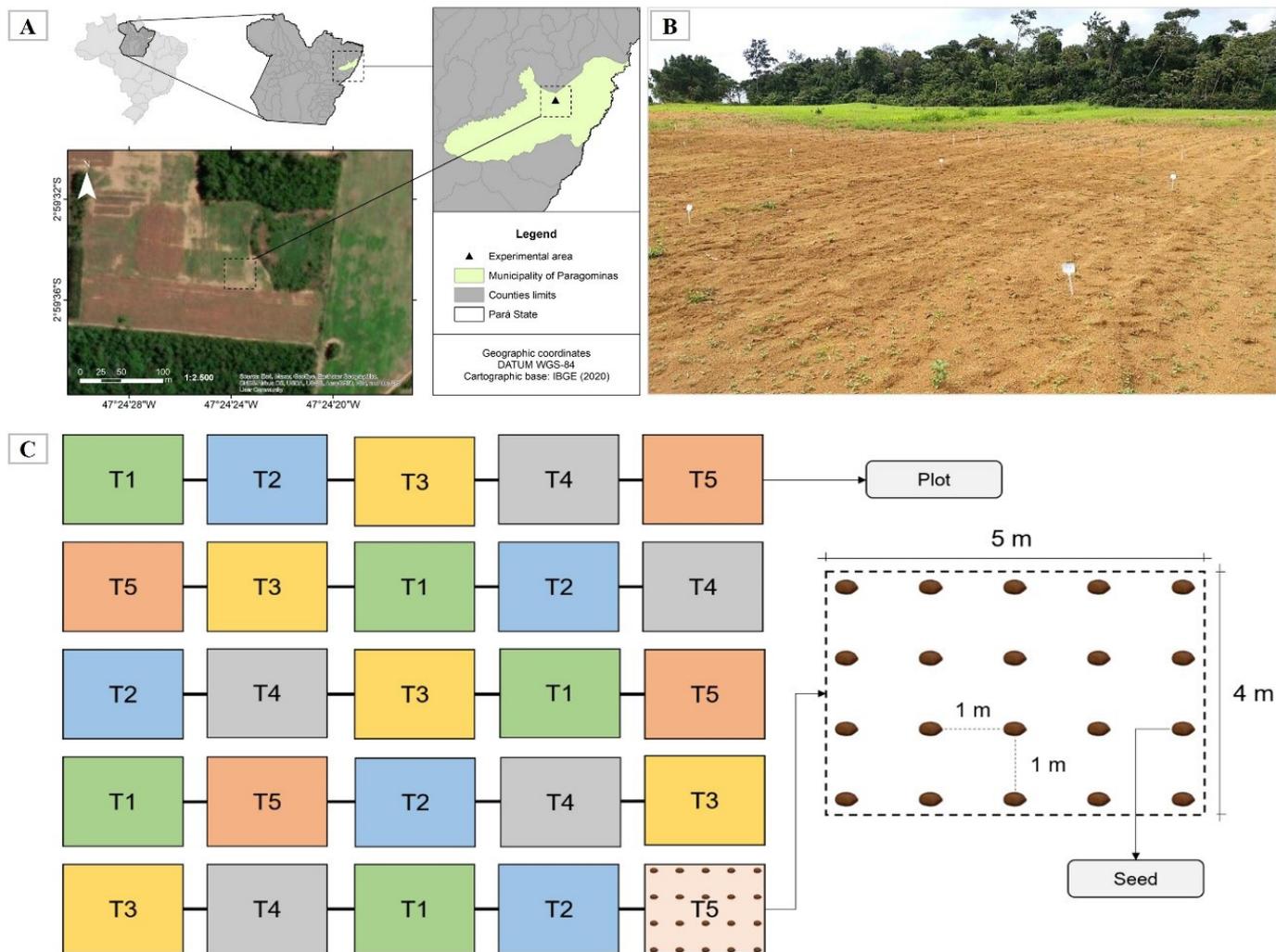


Figure 1. Location of the Embrapa Amazônia Oriental experimental field (A), planting area after soil preparation (B), and arrangement of seeding depth treatments (C) in the municipality of Paragominas, Southeast Mesoregion of the state of PA, North region of Brazil.

Table 1. Chemical attributes of the soil from experimental field of Embrapa Amazônia Oriental, in the municipality of Paragominas, state of Pará, North region of Brazil, 2021.

Characteristics	Results
pH (CaCl ₂)	5.60
Ca (cmol _c dm ⁻³)	3.70
Mg (cmol _c dm ⁻³)	1.10
Al (cmol _c dm ⁻³)	0.00
H+Al (cmol _c dm ⁻³)	2.00
K (cmol _c dm ⁻³)	0.24
Zn (mg dm ⁻³ ppm)	6.50
CEC	7.04
Base saturation	71.59%
Organic carbon (g dm ⁻³)	13.34
Organic matter (g dm ⁻³)	23.00

Experiment installation and conduction

The experiment was carried out for 120 days, starting with sowing in the field on March 11, 2021. The specie used was *S. parhyba* (*Schizolobium parhyba* var. *amazonicum*) For the execution of the experiment, a total of 400 seeds were used. The seeds were stored in polypropylene bags for 12 months,

in the Laboratory of Forestry Engineering of the Universidade do Estado do Pará. The environmental conditions were 28 °C and humidity of 85%.

The seeds were submitted to mechanical scarification in the opposite position to the hilum, to break tegumentary dormancy. Was used an electric sander and later, the seeds were immersed in water at environment temperature for 24 hours, according to the methodology described by the Rule for Seed Analysis (RAS) (Brasil, 2009).

The seeds were positioned horizontally in the soil, using one seed per hole, with a spacing of 1.0 × 1.0 m between holes (Figure 2), at different depths according to the treatments (Figure 1C). The holes were opened with the use of garden spatulas, and the respective depths checked with a millimeter ruler.

For the emergence evaluation, the number of germinated seeds was counted daily until the stabilization of the process. The seed that presented exposed cotyledons was considered as germinated.

The emergence percentage was determined by the number of germinated seeds in relation to the total number



Figure 2. (A) Seeds of *S. parahyba* (*Schizolobium parahyba* var. *amazonicum*) used in the experiment, (B) opening of pits in the experimental field of Embrapa Amazônia Oriental, and (C) open pits for sowing of the seeds at different depths (5, 10, 15, and 20 cm).

of seeds in each treatment. The average emergence time (AET) was determined from the daily count of the number of germinated seeds, which represents the weighted average of the time required for emerged ([Equation 1](#)):

$$AET = \frac{E_1 T_1 + E_2 T_2 + \dots + E_n T_n}{E_1 + E_2 + \dots + E_n} \quad (1)$$

where: AET - average time, in days, necessary to achieve the maximum emergence; E - number of emerged seeds; and, T - time.

The emergence speed index (ESI) was determined from the daily count of the number of germinated seeds, using the [Equation 2](#):

$$ESI = \frac{E_1 + E_2 + \dots + E_n}{D_1 + D_2 + \dots + D_n} \quad (2)$$

where: ESI - emergence speed index; E - number of emerged seeds computed in the counts; and, D - number of days elapsed from sowing from first to last count.

To assess survival, the number of surviving individuals was counted, and the survival rate was defined as the ratio between the number of surviving plants and the total number of initial seedlings. Verification of the number of surviving seedlings was performed at 120 days.

After 120 days the initial growth was evaluated. Five plants were randomly selected in each plot. The variables were: collar diameter (CD) determined with a digital paquimeter, number of leaves (NL) from the count of normal leaves in the seedling, and height (H) determined from the base to the apical bud, aided by a millimeter ruler. The slenderness degree, also known as the height/diameter ratio was obtained through the relationship between aerial part height (cm) and collar diameter (mm) ([Schwartz et al., 2022](#)).

Data analyses

Data on emergence, survival and variables as a function of initial growth were submitted to regression analysis. The

maximum technical efficiency (MET) was calculated from the equation $y = ax^2 + bx + c$. For all variables, the mathematical model $MTE = -b/2a$ ([Tiesdale et al., 1993](#)).

Results and Discussion

Emergence and survival

The emergence of seeds of *S. parahyba* started 2 days after sowing (DAS), with its maximum emergence point at 6 DAS, and at 8 days started the stabilization of emergence ([Figure 3A](#)). The seeds sown at 20 cm depth emerged 6 days after sowing and obtained a emergence rate greater than 70% and a survival rate of 77%. Based on regression analysis, were observed two quadratic functions, with a 99% confidence level for emergence, and an 88% confidence degree for survival. There was a quadratic response of percent seedling emergence to increasing seeding depth. The quadratic fit, $Y = -0.17x^2 + 3.23x + 77.25$, gave a maximum for percent seedling emergence of 92.6% at a seeding depth of 9.5 cm. The quadratic fit, $Y = -0.0986x^2 + 2.0548x + 75.909$, gave a maximum for seedling survival of 86.6% at a seeding depth of 10.4 cm ([Figure 3B](#)).

Seed emergence and seedling growth are affected by many environmental factors such as habitat disturbance. Sand burial at different depths change the temperature, moisture, and light conditions around the seeds, subsequently affecting seed emergence and seedling growth ([Teixeira et al., 2016](#); [Zuo et al., 2016](#)). Sand burial can adjust the emergence time of seeds ([Ye et al., 2019](#)), and can maintain high humidity, thus preventing the seeds directly exposed to the soil surface from being damaged at high temperature or low temperature, increasing the seedling growth speed ([Tao et al., 2022](#)). The results of the current study showed that sowing depth had significant effects on the first emergence time, peak emergence time, emergence rate seedling growth height of *S. parahyba* seeds.

With the except for depth of 20 cm, the seedlings of *S. parahyba* presented excellent emergence and survival rates at other depths ([Figure 3B](#)). It is established that the seed requires moisture and suitable temperature to germinates. The low

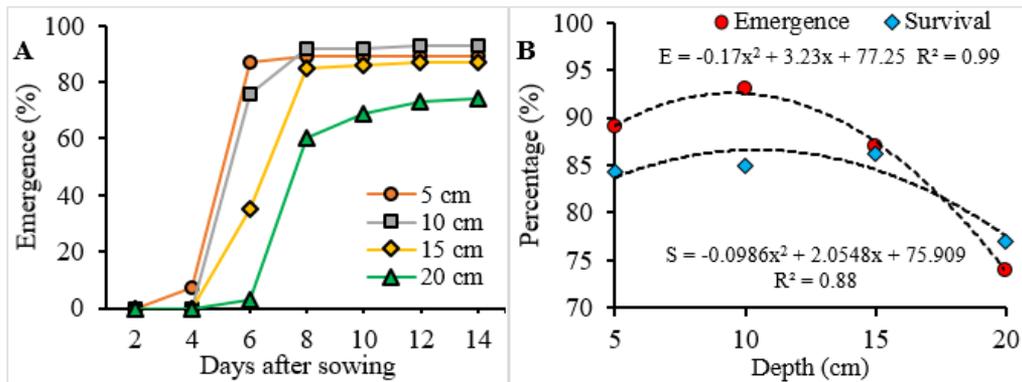


Figure 3. Accumulated emergence (A) and seed emergence at 13 days and seedlings survival at 120 days (B) of seeds of *S. parahyba* (*Schizolobium parahyba* var. *amazonicum*) under different depth levels (5, 10, 15, and 20 cm), in the municipality of Paragominas, state of Pará, Brazil.

water availability due evaporation and extreme temperature fluctuations, which occurs at the soil surface, limited the chances of a seeds of *S. parahyba* to germinate. Surface seeds are exposed to excess or water deficit, in addition to climatic variations, which may have resulted in low seed emergence observed throughout the experiment (Gehling et al., 2017). Seeds at a high depth, such as the 20 cm depth evaluated in this study, tend to have greater difficulty in breaking the soil physical barrier, a fact refuted by the high emergence rates found in this depth level with seeds of *S. parahyba*, confirming that the species have different characteristics regarding their survival in the field.

It is worth mentioning that, despite the high emergence and survival rates, and regardless of sowing depth, it is necessary to carry out pre-emergence treatments, due to the presence of tegumentary dormancy in the seeds of *S. parahyba* (Carvalho et al., 2019; Fernandes et al., 2019). Another fact observed during the execution of the experiment, that may have interfered in the results of seed emergence and seedling survival was the attack of animals/insects on the seeds. Gehling et al. (2017), affirmed that seeds at a lower depth become easy targets for predators.

In the evaluation of the average time and emergence speed of seedlings of *S. parahyba* var. *amazonicum* from regression analysis, linear responses were obtained as a function of different sowing depths (Figure 4).

The average emergence time (AET) fitted an increasing linear function with increasing depth, with a confidence level of about 99%. The lowest AET was obtained at the depth 5 cm and the highest was obtained at a depth of 20 cm. At depths 5, 10, and 15 cm, the AET required for emergence was 5, 6, and 7 days, respectively. It is generally established and accepted that deep sowing affects seedling emergence. The sowing depth affects plant emergence speed. The emergence of seedlings of *Mimosa bimucronata* (DC.) Kuntze., planted at greater depths, occurred later than those planted close to the soil surface (Berto et al., 2014).

As for the emergence speed index (ESI), it decreased with an increase in sowing depths. The degree of confidence of the linear function for this variable was 90%. It was observed

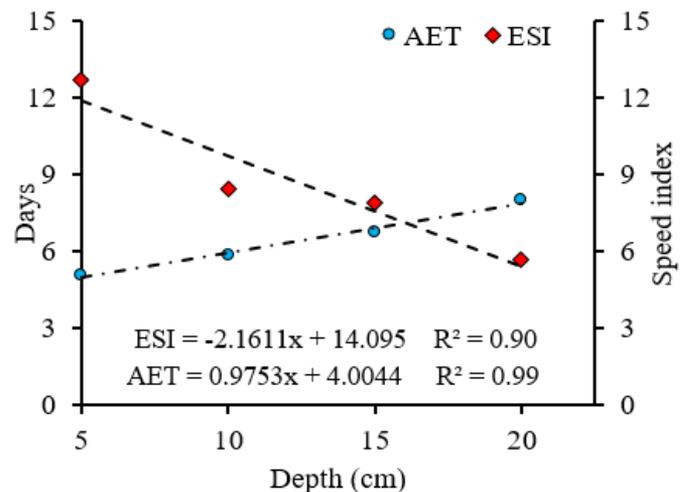


Figure 4. Average emergence time (AET) and emergence speed index (ESI) of seeds of *S. parahyba* (*Schizolobium parahyba* var. *amazonicum*) at different sowing depths, in the municipality of Paragominas, state of Pará, Brazil.

that the depth of 5 cm provided better result of ESI, 13 of speed, while the other depths provided a reduction of it. The increased the sowing depth results in a delay in seedling emergence (Silva et al., 2018). The *Erythrina verna* (Mulungú) seeds, planted at different soil depths, showed better results at 1 and 3 cm deep (Pêgo et al., 2015).

The decrease in emergence speed as a function of increasing sowing depth, may be related to temperature variations throughout the day, these variations favor seeds placed at lower depths (Cardoso et al., 2008).

The increase in the physical barrier in deeper sowings delays the emergence of seedlings and, consequently, the seedlings become more sensitive to environmental conditions, impairing their initial stage of development. Very shallow seeding can lead to a high incidence of spider roots (Roy et al., 2003) which develop horizontally just below the soil surface, and which are of very low quality. However, provided the radicle of the germinating seed penetrates the soil, the seedling will develop normally with a tap root (Proctor & Sullivan, 2013) Too shallow seeding, i.e., less than 5 cm and

not included in these studies, may lead to desiccation of the seedlings and low plant populations. Even if shallowplanted seeds survive and germinate, there is the likelihood that the resultant seedlings will scavenge for water and mineral nutrients horizontally and not penetrate deeply into the soil. Such behavior leads to the formation of low quality 'spider' roots as described and illustrated in [Roy et al. \(2003\)](#). This plant behavior, known as 'plasticity' is relatively common in plant species particularly in stress situations ([Hutchings & Kroon, 1994](#)).

Initial growth

The regression analysis, adjusted to a linear function, with a confidence level of about 74% ([Figure 5A](#)). It was found that the seedlings of *S. parahyba* reached the higher height (H) at 5 and 10 cm depths (30.82 and 27.54 cm, respectively), 120 days after sowing ([Figure 5A](#)). The collar diameter (CD) was adjusted to the quadratic regression model, with a confidence level of 82%. The CD of the seedlings of *S. parahyba* decreased from the depth of 10 cm. This tendency of reduction becomes the main factor to understand the levels of tolerable depth for the seedling, aiming its development ([Figure 5B](#)).

The curve presents the number of leaves obtained as a function of depth, thus generating a graph of quadratic function, with a confidence level of 83%. It was observed that plants sown the depth 20 cm presented the lowest value of leaves per plant, with average of 19.2 leaves ([Figure 5C](#)). The slenderness degree (DS), or height/diameter ratio of seedlings

of *S. parahyba* var. *amazonicum* showed little difference between treatments, with averages varying between 3.38 and 4.10 ([Figure 5D](#)), with quadratic regression adjustment, with a reliability degree of 73%.

From the depth of 10 cm, the increase in sowing depth provided a decrease in the aerial part height. The height of the aerial part of *S. parahyba* was greater in plants sown at 20 cm depth, which reached 33.67 cm ([Gomes et al., 2021](#)). The authors concluded that sowing depth did not interfere with plant height. *Acacia polyphylla* DC seeds, planted at 1.4 and 2.8 cm deep, and *Peltophorum dubium* (Sprengel) Taubert seeds, planted at 1 and 3 cm deep, showed the best results for emergence rate ([Rodrigues et al., 2016](#); [Zuffo et al., 2020](#)).

Deep seed sowing has a number of effects on seedling growth. For instance, there may be an increase in the time between seed emergence and seedling emergence ([Li, 1997](#)), which largely determines the ranking of seedlings in the competitive hierarchy for growth resources. An increase in hypocotyl or epicotyl length, as noted in deep seeding, will reduce the probability of the seedlings being capable of overcoming soil strength ([Parker & Taylor, 1965](#)) and render the seedlings more susceptible to attack by pathogens.

The plants of *Zizyphus joazeiro* Mart. (Juazeiro) showed a reduction of 1.22 cm in seedling length, for each centimeter of sowing depth in the soil ([Alves et al., 2008](#)). Probably, the seeds energy cost for emergence at excessive depths affects seedling development. The increasing depth causes a greater effort by the seedling to break the physical barrier ([Alves et](#)

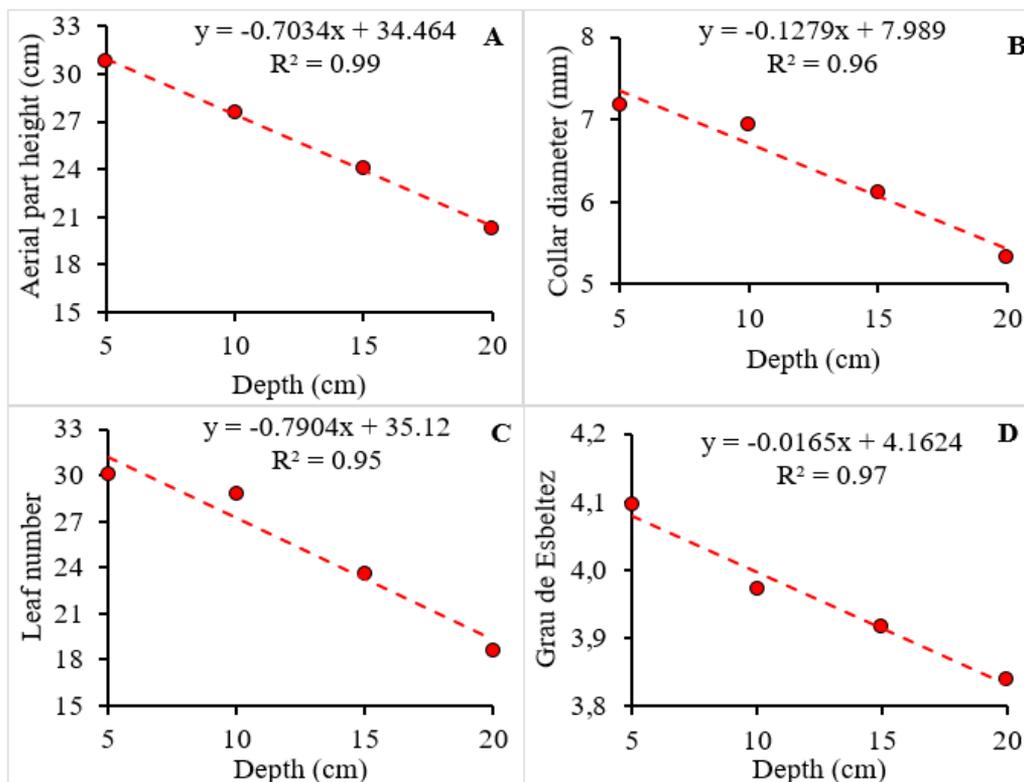


Figure 5. Aerial part height (A), collar diameter (B), number of leaves (C), and slenderness degree (D) of seedlings of Parica (*Schizolobium parahyba* var. *amazonicum*) at different depths at 120 days after sowing in the field, in the municipality of Paragominas, state of Pará, Brazil.

al., 2013). This physiological wear and tear caused by the physical impediment to emergence results in plants with smaller diameter and height.

Regarding the variable of collar diameter (Figure 5B), there is a variation in the values found, with treatment T2 (5 cm) having the highest mean (7.18 mm) between treatments. The depth of 5 cm proved to be favorable for the development of the collar diameter of the plants, and is directly related to the aerial part height, as the balance of seedling development is observed (Araújo et al., 2017). As it is a heliophilous species, *S. parahyba* tends to have a greater initial development when placed in full sun, and the CD becomes a considerable morphological parameter, aiming to verify the adaptation of the species in the field.

Regression analysis indicates a significant difference between the number of leaves and seeding depth. The greater the sowing depth, the smaller the number of leaves (Figure 5C). The number of leaves, in the initial growth of the species, decreases when submitted to water deficit (Nascimento et al., 2011; Duarte et al., 2020).

The number of leaves per plant is directly linked to its development, since it is through them that chemical reactions such as photosynthesis and transpiration occur. Thus, it was observed that the seedlings with greater development in the field had the highest number of leaves per plant, with an average of 30 leaves being measured for a depth of 5 cm, 29 leaves for a depth of 10 cm and 24 leaves for a depth of 15 cm (Figure 5C).

The slenderness degree is an index adopted to infer about the quality of seedlings, and this relationship reflects the accumulation of reserves, resistance and fixation of the plant in the soil. The seedlings are considered of excellent quality when the height/diameter ratio is greater than 10 (Rossa et al., 2013). In the present work all treatments presented results lower than the reference value. Seedlings of *Schizolobium parahyba* var. *amazonicum*, cultivated in different substrates, obtained the minimum reference value for the degree of slenderness only at 103 days after sowing (Barbosa et al., 2019).

Regarding the determination of the maximum technical efficiency (MTE) for the variables studied, after adjusting the polynomial regression equations, the following results were obtained, presented in Table 2. The results found by the regression analysis, suggest the use of sowing at a depth of 9.5 cm and 10.4 presented the best values of emergence (92.6%) and survival (86.6%), respectively. For all variables in which quadratic functions were fitted, the treatment T3 (10 cm) was shown to be the maximum point of the regression curves, indicating a decrease in the results from the depth of 15 cm, although the depths of 5 and 15 cm obtained satisfactory results. The maximum technical efficiency presented that the ideal depth for the evaluated parameters is between of 9.5 cm and 10.4 cm, with an average of 9.9 cm, suggesting that the ideal sowing depth for *Schizolobium parahyba* var. *amazonicum* is approximately 10 cm deep (Table 2).

Table 2. Maximum technical efficiency (MTE) obtained from the quadratic equations of emergence, survival, initial growth, and degree of slenderness at different sowing depths of seeds of *Schizolobium parahyba* var. *amazonicum*.

Variables	MTE (x)	MTE (y)
Emergence (%)	9.5	92.6
Survival (%)	10.4	86.6
Average	9.9	89.6

The ideal depth for sowing *S. parahyba* seeds is 10 cm (Gomes et al., 2021), which reinforces the result obtained in this study. This good result is justified, as this depth provides the ideal level of oxygen and temperatures for the seed, in addition to facilitating the absorption of nutrients by the roots, ensuring the support of the plant. Direct sowing, in addition to good emergence rates, is recommended for the lowest installation cost (Pellizzaro et al., 2017; Figueiredo et al., 2021), because, generally, the costs are 30 to 38% cheaper than the conventional seedling production system (Grossnickle & Ivetić, 2017). The costs with direct seeding at 1 year of age were up to 2.5 times less expensive than the seedling model, using a density of 1089/ind./ha, which represents an alternative method for the restoration of degraded areas of forest species (Palma & Laurance, 2015; Raupp et al., 2020).

Conclusions

The emergence percentage was higher at a depth of 10 cm, reaching 93% of the germinated seeds. The best result for the average time and emergence speed index were obtained at depth of 5 cm.

The depth of 10 cm presented the best result for the survival rate and initial growth of *S. parahyba*.

The ideal seeding depth for the emergence, survival and initial growth of *S. parahyba* is at a depth of 10 cm.

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Compliance with Ethical Standards

Author contributions: Conceptualization: LFSD, JNS, HDS; Data curation: JNS, HDS; Formal analysis: LFSD, JNS, HDS, AS, JCOJ; Investigation: LFSD, JNS, HDS, JCOJ, AMC, AS, JCOJ; Methodology: LFSD, JNS, HDS; Writing – original draft: LFSD, JNS, HDS, JCOJ, AMC, AS, JCOJ; Writing – review & editing: LFSD, JNS, HDS, JCOJ, AMC, AS, JCOJ.

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